Pestic. Sci. 1996, **47**, 235–240

Aerobic Metabolism of Flupropacil in Sandy Loam Soil

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(Received 13 February 1995; revised version received 8 January 1996; accepted 1 February 1996)

Abstract: The aerobic soil metabolism of [^{14}C]flupropacil (isopropyl 2-chloro-5-(1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-trifluoromethylpyrimidin-1-yl)benzoate) was determined in microbially active, sieved (2-mm) sandy loam soil with a soil moisture content of 75% at 1/3 bar. The soil was treated with [^{14}C]flupropacil at 0·5 mg kg^{-1} (twice the field use rate) and placed in incubation flasks connected to a series of traps (50 g litre^{-1} NaOH, 0·5 M H_2SO_4, ethylene glycol) and incubated at 25(± 1)°C. Soil was sampled at 0, 3, 9, 20, 30, 48, 76, 120, 181 and 238 days of aerobic incubation. Volatiles were collected once every two weeks and on the day of soil sampling. Flupropacil metabolized with a half-life of 79 days under aerobic conditions. The major metabolite was flupropacil acid which accounted for up to 69·1% of the initially applied radioactivity at Day 238. Each of the two minor metabolites detected at the end of the study accounted for less than 0·5%. One of the minor metabolites was identified as C4242 acid (2-chloro-5-(1,2,3,6-tetrahydro-2,6-dioxo-4-trifluoromethylpyrimidin-1-yl)benzoic acid). Only a negligible portion (less than 0·3%) of the applied flupropacil was mineralized to [^{14}C]carbon dioxide. Extractable radioactivity ranged from 78·9% to 95·5%, with bound residues accounting for 3·2%–23·4%. The material balance ranged from 91·6% to 104·4%.

Key words: aerobic, soil, metabolism, flupropacil

1 INTRODUCTION

Flupropacil (isopropyl 2-chloro-5-(1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-trifluoromethylpyrimidin-1-yl)-benzoate; UCC-C4243) is an experimental uracil herbicide that is used for controlling a wide variety of broadleaf and some annual grass weeds.[1,2] The mode of action of this herbicide is inhibition of the enzyme protoporphinogen oxidase. Microbial degradation plays an important role in the degradation and final mineralization of herbicides.[3] Microbial degradation of pesticides in soils is important, not only to predict the behavior of the pesticides, but also to determine the nature and extent of formation of its metabolites to which rotational crops and non-target organisms will be exposed.

This study was designed following US EPA Subdivision N (162-1) guidelines[4] for pesticide registration and/or reregistration. This study determines the metabolism of flupropacil and the nature of its metabolites, under aerobic conditions in a microbially active sandy loam soil. Since no information is available on the metabolism of flupropacil under aerobic conditions, the information obtained from this study can be used to predict the fate of flupropacil and its metabolites in the environment, under actual field use.

2 MATERIALS AND METHODS

2.1 Test substance

[2-^{14}C-*Pyrimidine*]flupropacil (radiopurity 99%; specific activity 30 mCi mmol^{-1}), synthesized by ChemSyn Science Laboratories (Lenexa, KS), and nonlabeled flupropacil and its metabolites (Fig. 1), synthesized by Uniroyal Chemical synthesis group (Middlebury, CT) were used in this study.

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Pestic. Sci. 0031-613X/96/$09.00

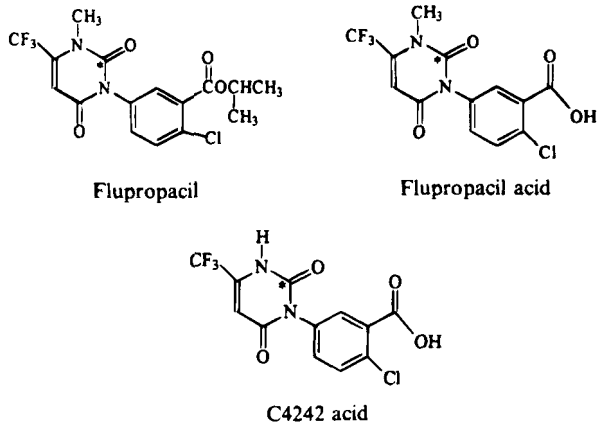


Fig. 1. Structures of flupropacil and two of its metabolites. The asterisk indicates position of radiolabel

2.2 Radioanalysis

Quantitative radioactivity measurements were made on a Beckman LC 60001C liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA) using Packard 'Ultima Gold'™ scintillation cocktail (Packard Instrument Company, Meridan, CT). Bound residues were determined by combustion of air-dried samples in a Packard 306 biological oxidizer. The [^{14}C]carbon dioxide produced was absorbed using Carbo-Sorb^{R} (Packard) and mixed with Perma-fluor^{R} for liquid scintillation counting (LSC).

2.3 HPLC analysis

HPLC was the primary method of analysis for identification of flupropacil and its metabolites. HPLC analysis was performed on a Hitachi LC with L-5000 controller, L-3000 UV detector (254 nm) and a Berthold (Germany) LB 506 solid cell radioactive monitor (RAM). The UV and RAM detectors were connected in series directly from the output of the HPLC column. The column used was Phenomenex C18, 10 μm Bondex (30 cm × 4·6 mm). A linear solvent gradient system, as shown in Table 1, was used with buffered water (0·025 M triethanolamine, pH adjusted to 4·0 with formic acid) and acetonitrile (solvent B).

Aliquots (10–15 ml) of each pooled extract were concentrated to less than 1 ml under a gentle stream of nitrogen. The volume was then adjusted to 1 ml using acetonitrile, the sample was vortexed, filtered through a 0·25-μm filter, and triplicate 100-μl aliquots were analyzed by LSC. An additional 30 μl of each concentrated extract was analyzed by HPLC, and 1-min fractions were collected and analyzed by LSC for quantitation of residues. The relative retention time was 41·9 min for flupropacil, 19·5 min for flupropacil acid and 16·6 min for C4242 acid. The unknown metabolite eluted at 4·7 min.

2.4 Thin-layer chromatography

Two-dimensional thin-layer co-chromatography (TLC) was used as a confirmatory analytical procedure for the identification of [^{14}C]flupropacil and its metabolites. TLC plates (silica gel 60F$_{254}$, 20 × 20 cm, 0·2 mm thickness from EM Science) were spotted with aliquots (10–20 μl) of soil extracts and [^{14}C]flupropacil and its metabolite standards. The plates were then developed in the first dimension using chloroform + methanol + acetic acid (95 + 5 + 1 by volume) and in the second dimension in methanol + water (8 + 2 by volume).

The developed TLC plates were visualized using a linear image analyzer (AMBIS Radioanalytic Imaging System) and under a UV (254 nm) lamp. The R_f values in the first and second dimension for flupropacil were 0·81 and 0·61, respectively, for flupropacil acid 0·4 and 0·80, respectively and for C4242 acid, 0·24 and 0·94, respectively.

2.5 Test soil

Paxton sandy loam soil used in this study was obtained from Middlebury, CT. The soil organic content was

TABLE 1
HPLC Solvent Gradient System

Time (min)	*Solvent A*[a] (%)	*Solvent B*[b] (%)
0	95	5
30	50	50
35	50	50
40	5	95
45	5	95
50	95	5
65	95	95

[a] 0·025 M Triethanolamine in water.
[b] Acetonitrile.

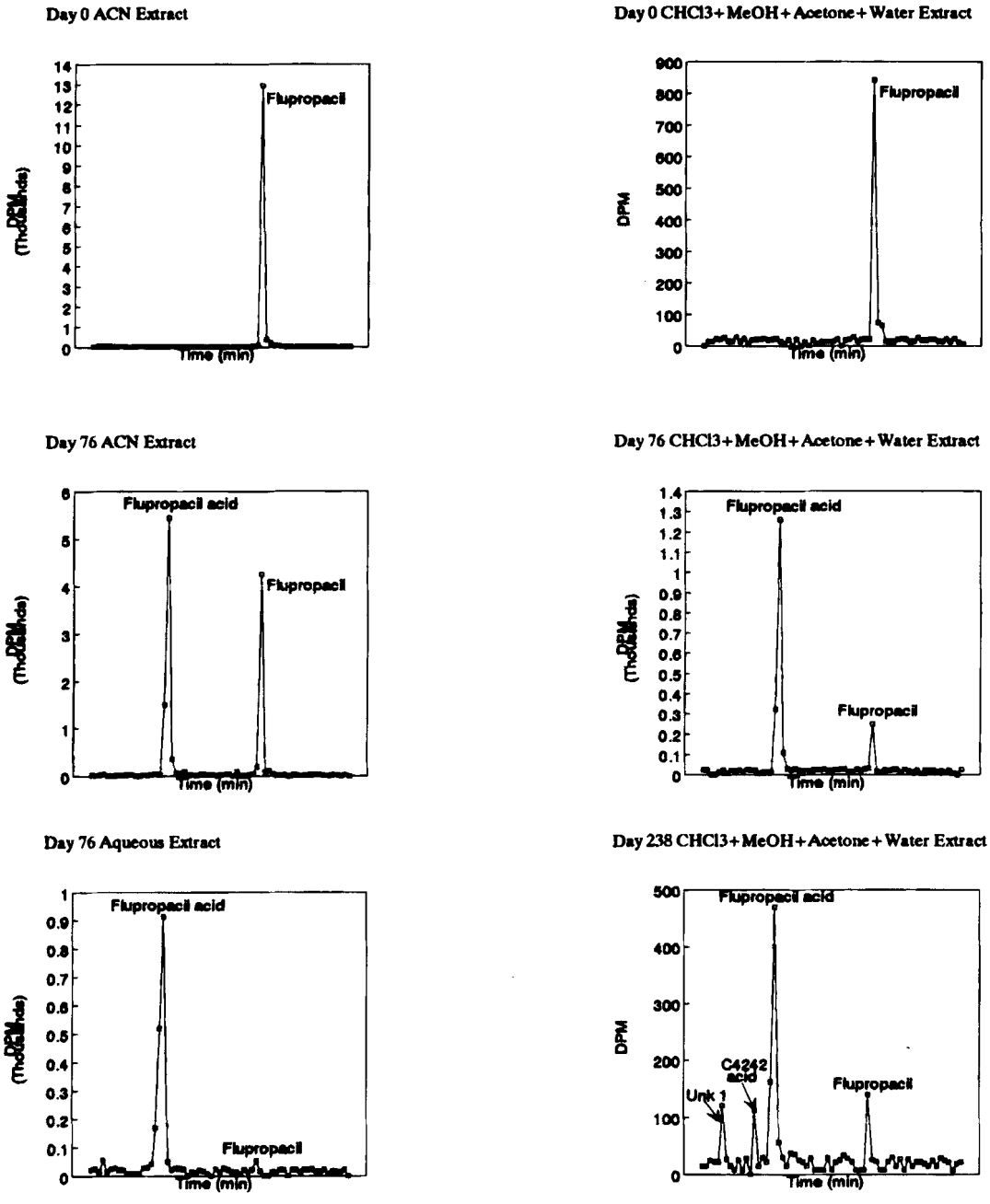


Fig. 2. HPLC analyses of soil extracts

4·8% and pH 6·3. The soil viability was tested prior to its use by microbial plate count, and was found to be viable. The soil was sieved (2-mm) and the soil moisture content was adjusted to 75% at 1/3 bar. Soil characterization[5] following USDA soil textural analysis is provided in Table 2.

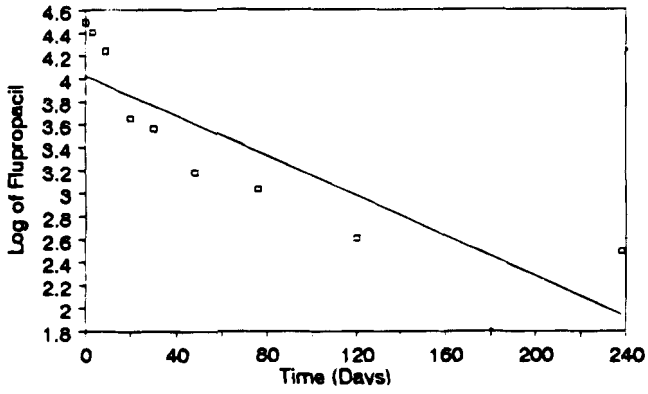
2.6 Soil incubation

Approximately 20 g (dry weight equivalent) of sieved (2-mm), moist soil was weighed into each of the 28 pre-weighed 500-ml incubation flasks. The incubation flask is equipped with air inlet and outlet ports each fitted with a stopcock. Aliquots (110 μl) of 0·09 mg ml^{-1} of [^{14}C]flupropacil in acetonitrile were added evenly to the soil in each flask, using a syringe. The 0·5 mg kg^{-1} application rate selected was twice the actual field use

TABLE 2
Paxton Sandy Loam Soil Characterization

Sand (%)	*Silt* (%)	*Clay* (%)	*pH*	*Organic matter* (%)	*Bulk density* (*g cm*$^{-3}$)	*FMC*[a] (% *at 1/3 bar*)
70	24	6	6·3	4·8	1·27	25·0

[a] % Field moisture capacity.



Time (days)	*Applied flupropacil (%)*	*Ln*	*Fitted log*
0	89·2	4·4912	4·0178
3	81·9	4·4050	4·0004
9	69·7	4·2439	3·9479
20	38·6	3·6530	3·8519
30	35·3	3·5642	3·7645
48	24·1	3·1830	3·6073
76	20·8	3·0354	3·3627
120	13·6	2·6086	2·9783
181	9·5	2·2492	2·4455
238	12·2	2·4990	1·9476

Regression output

Constant	4·0266
Std error of *Y* est	0·4052
R squared	0·7791
No. of observations	10
Degree of freedom	8
X coefficient(s)	−0·0087
Std error of coef	0·0016
Half-life of flupropacil	79·3 days

Fig. 3. Half-life determination of flupropacil

rate. The soil in two of the 28 flasks was left untreated and these flasks served as controls, for monitoring the soil moisture content. In addition, an aerobic/anaerobic strip was placed in each of the two control flasks for monitoring the aerobicity of the test system. The soil moisture content was maintained at approximately 75% at 1/3 bar throughout the study period. The incubation flasks were then placed in a Hotpack (Model 352602, Philadelphia, PA) incubator maintained at 25(±1)°C throughout the study period.

Once every two weeks and just prior to sampling, each flask was connected to a series of traps, (50 g litre^{-1} sodium hydroxide solution, 0·5 M sulfuric acid and ethylene glycol; 15 ml each) and flushed with CO_2-scrubbed humidified air for approximately 5 min, using a vacuum pump. All traps were replaced with fresh trapping solutions after flushing.

Duplicate soil samples were taken immediately after application and were treated as Day 0 samples. Additional duplicate soil samples were taken on Days 3, 9, 20, 30, 48, 76, 120, 181 and 238.

2.7 Extraction and/or analysis

Soil from the incubation flask was transferred into a polypropylene Nalgene bottle. The soil adhering to the walls of the flask was washed with acetonitrile (15–20 ml), and the wash was transferred to the Nalgene bottle. This procedure was repeated twice more. The soil was then extracted by sonication (Fisher Scientific Solid State/Ultrasonic FS-14 Sonicator), for approximately 15 min. Following the extraction, the sample was centrifuged (Beckman J2-21) for 10 min at

TABLE 3
Distribution of Radioactivity from [^{14}C]Flupropacil Residues in Soil

Incubation time (days)	*Extractable*			*Bound* (%)	*$^{14}CO_2$* (%)	*Recovered* (%)
	Ext 1[a] (%)	*Ext 2*[b] (%)	*Ext 3*[c] (%)			
0	89·6 (±0·4)	4·7 (±0·0)	NA[d]	4·2 (±1·0)	NA	98·4 (±1·4)
3	82·6 (±2·0)	7·0 (±0·5)	NA	7·1 (±1·7)	ND[e]	96·7 (±0·7)
9	83·3 (±1·0)	12·2 (±1·6)	NA	6·0 (±1·4)	ND	101·5 (±2·0)
20	63·8 (±1·5)	24·1 (±2·5)	NA	9·4 (±1·8)	ND	97·2 (±2·7)
30	58·2 (±1·0)	20·4 (±0·0)	9·9 (±1·3)	3·2 (±0·1)	ND	91·6 (±0·4)
48	60·3 (±2·5)	26·9 (±3·7)	NA	8·9 (±0·8)	ND	96·1 (±1·9)
76	57·4 (±0·1)	16·4 (±0·6)	14·6 (±0·1)	10·2 (±1·2)	ND	98·6 (±1·2)
120	57·7 (±0·9)	14·3 (±0·1)	17·3 (±0·2)	15·1 (±1·7)	ND	104·4 (±2·7)
181	68·5 (±0·7)	5·7 (±0·2)	6·4 (±0·2)	23·4 (±0·7)	0·3±	104·2 (±0·9)
238	63·5 (±0·9)	7·8 (±0·2)	7·9 (±0·5)	15·0 (±4·9)	0·1±	94·0 (±5·2)

[a] Acetonitrile.
[b] Chloroform + methanol + acetone + acetic acid (3 + 3 + 3 + 1 by volume).
[c] 0·025 M triethanolamine in water (pH adjusted to 4·0 with formic acid).
[d] Not analyzed.
[e] Not detected.

10 000 rev min^{-1}. The supernatant was decanted into a stoppered graduated cylinder and the volume was recorded. Triplicate aliquots (100 µl) of the extract were analyzed by LSC. The extraction was repeated twice more, the three acetonitrile extracts were pooled and the final volume was recorded. Triplicate aliquots (100 µl) of the pooled extract were analyzed by LSC.

Following the acetonitrile extraction, the soil was extracted by sonication with chloroform + methanol + acetone + water (3 + 3 + 3 + 1 by volume; 15 ml). The extraction and analysis were similar to that described above. The extraction was repeated once more. The two chloroform + methanol + acetone + water extracts were pooled, the final volume was recorded, and triplicate aliquots (100 µl) were analyzed by LSC.

TABLE 4
HPLC Analysis of Soil Extracts

Incubation time (days)	*Flupropacil* (%)	*Flupropacil acid* (%)
0	89·2	ND[a]
3	81·9	4·8
9	69·7	20·1
20	38·6	48
30	35·3	40·9
48	24·1	60·7
76	20·8	66·0
120	13·6	68·3
181	9·5	60·0
238	12·2	69·1

[a] Not detected.

Due to the increase of bound residues with time, all soils sampled after Day 48 were further extracted twice with a third solvent, an aqueous buffered solution (0·025 M triethanolamine in water, pH adjusted to 4·0 with formic acid). The extraction and analysis were similar to that described above.

Duplicate aliquots (approximately 200 mg) of the extracted soil were then air-dried and combusted (Packard 306 oxidizer) for bound residues.

Aliquots of the trapping solutions were analyzed directly by LSC. Additional aliquots (10–20 ml) of the 50 g litre^{-1} sodium hydroxide trapping solution from selected sampling times were taken and 5–10 mg of barium chloride were added. The samples were mixed by vortexing and centrifuged at 10 000 rev min^{-1} for 10 min. Aliquots (1 ml) of the supernatant were analyzed in triplicate by LSC.

3 RESULTS AND DISCUSSION

Flupropacil was microbially degraded with a half-life of 79 days in sandy loam soil. The material balance ranged from 91·6% to 104·4%. Extractable radioactivity ranged from 78·9% to 95·5% and bound residues accounted for 3·2–23·4%. ^{14}C-Volatile radioactivity was detected only in the 50 g litre^{-1} sodium hydroxide trap and totaled less than 0·3% by the end of the study. The volatile radioactivity was identified as [^{14}C]carbon dioxide using barium chloride to precipitate this and form barium carbonate. The distribution of radioactivity is provided in Table 3. All data are expressed as of applied radioactivity.

Flupropacil decreased to 12·2% by the end of the study. Only one major metabolite was detected and was identified as flupropacil acid by HPLC Analysis and confirmed by 2-D TLC analysis. Flupropacil acid is fairly stable and accounted for up to 69·1% by Day 238. Two minor metabolites were detected at Day 238 only (Fig. 2), and each accounted for less than 0·5% of the applied radioactivity. One of the minor metabolites was identified as C4242 acid (2-chloro-5-(1,2,3,6-tetrahydro-2,6-dioxo-4-trifluromethylpyrimidin-1-yl)benzoic acid), and the other minor metabolite could not be identified and is referred to as unknown. The results of the HPLC analysis for flupropacil and flupropacil acid are provided in Table 4. Reconstructed chromatograms from HPLC fraction-collection analysis of selected soil extracts are provided in Fig. 2.

4 CONCLUSIONS

Results from our study show flupropacil to degrade fairly rapidly in soil, with a half-life of 79·3 days (Fig. 3). The rapid degradation of flupropacil was similar to that of uracil,[6] and other substituted uracil compounds.[7] The major metabolite formed in this study was flupropacil acid, which is a hydrolysis product. One of the commonly occurring metabolic processes in microorganisms is hydrolysis. Hydrolysis of the ester, halide, ether and amide bonds is common, as seen in herbicides such as carbaryl,[8] chlorinated pesticides and organophosphates such as diazinon.[9] Although the adsorption of flupropacil on various soil types[10] tested is low and it may therefore leach through soil, its short half-life under controlled conditions, as shown in this study, and the rapid degradation under field conditions,[2] indicate that flupropacil will not persist long in the environment to leach. The degradation of flupropacil applied to glass slides and irradiated under a UV lamp shows the formation of three photoproducts, which were not identified.[2] This suggests that, although the major metabolite, flupropacil acid, appears to be persistent under controlled aerobic conditions, its behavior may be different in the environment where various degradation processes take place simultaneously.

ACKNOWLEDGEMENT

We wish to thank Dr Hamdy M. Balba for his encouragement and continuous support and the crop protection department management, for allowing us to publish this work.

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